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ANTIMICROBIAL EFFECTS OF STEM AND FRUITS EXTRACT OF *PROSOPIS CINERARIA* (L) DRUCE

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Abstract: Extensive and uncontrolled widespread use of antibiotics has developed the emergence of resistance by a variety of pathogens against several classes of antibiotics. The scientists are in continuous search of some valuable plants to overcome the problem of antimicrobial resistance. *Prosopis cinerarium is* extensively employed in the treatment of boils, skin disorders, as blood purifier and for dysentery etc. The objective of this research was to explore and identify the antimicrobial activity of aqueous-ethanolic extract of stem and fruits of *Prosopis Cineraria* (L) druce. Antimicrobial activity of both of the extracts was evaluated by disc diffusion assay against three gram positive bacteria, three gram negative bacteria and two fungal isolates. IC50 and IC90 for all bacterial cultures were determined by using 96-well plate method. Results indicated that both stem and fruit extracts inhibit all the bacterial and fungal cultures up to a considerable level. Stem extract exhibited greater activity than fruit extract and both extracts were found to be more effective against gram positive bacteria than that of gram negative bacteria. Preliminary phytochemical analysis revealed that it contained saponins, alkaloids, tannins, pholobatannins, flavonoids, steroids and glycosides which are responsible for antimicrobial activity. However, there is still a strong need of further studies to explore the molecular basis of mechanism of anti microbial action of plant extract.

Keywords: *Prosopis cineraria*, multi drug resistant bacteria, antimicrobial, ciprofloxacin, gentamicin.

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INTRODUCTION

Herbal medicines are the mainstay of treatment in 75-80% population, mainly in the developing countries. Reason for using herbal remedies as main source of treatment is that these have better acceptability due to economy and with no or only a few side effects [1,2]. Natural sources are most ancient method of treatment and approximately 25% of medicines have their origin from natural sources [3]. Substances derived from plants have become paramount importance currently because of their numerous functionalities [4]. Natural medicines are availing wide acceptance now a days, therefore scientist are interested in natural resources for discovering new medicinal agents [5]. Medicinal plants are considered as valuable potential source of new therapeutic entities [6-7]. In spite of so much progress in medical fields the infectious diseases are in progressing at a high speed [8]. Multiple drug resistant micro-organisms have come into existence due to imprudent use of antibiotics [9-11]. Therefore a need arise to develop new anti-microbial. Plants are rich source of anti-infective agents as their secondary metabolites like flavanoids, phenolics & polyphenols, terpenoid, sesquiterpenes and tannins are effective against a number of microorganisms [6]. Most of research work carried out till date for search of new antibacterial agent had mainly focused on bacteria and fungi and only a little amount of research has been done on higher plants [12].

Prosopis cineraria is considered as a tree or small shrub [13], which belongs to family Leguminosae and it is considered as largest family of flowering plants [14]. It is known as Jand in local language. It is distributed in various desert locations of Gulf countries and spread in different locations of India like Rajasthan, Gujarat, Haryana, Uttarpradesh and Tamilnadu [15].

Flowers of *Prosopis cineraria* are meshed with sugar in water and are used for skin disease, for boils prevention, as blood purifier and for producing a cooling effect. Dry pods of plants are also eaten by farmers to avoid excessive thirst [16]. Dry bark is used to combat the muscle tumors, piles, asthma, bronchitis, leucoderma, dysentery, tonic, leprosy, and as anthelmentic and for curing wandering mind as well as for its cooling effects and also effective in scorpion and snake bites [17]. The fruit of the plant is used as a vegetable traditionally known as sangari [18]. Plant is widely used as protecting to avoid miscarriage in pregnant women who eat the mixture of sugar and minced flowers of the plant [13,18-19]. Bark of plant has abortificent and laxative properties [20]. Bark is beneficial in cases of rheumatism and also used for scorpion stings. Pods of the plant have astringent action. Ashes prepared by burning the leaves have hair removing properties when applied on skin with rubbing [13,21].

MATERIALS AND METHODS

Collection and authentication of plant material: Stem and fruits of Prosopis cineraria were collected from Cholistan Institute of Desert Studies (SIDS) and authenticated from a botanist. A

portion of plant material was submitted in herbarium of the Faculty of Pharmacy and Alternative Medicine, the Islamia University of Bahawalpur and herbarium voucher number was obtained. Stem: PC-ST-03-12-037, Fruits: PC-FT-06-12-038.

Preparation of plant extract: Plant material was washed under running tap water and then by distilled water to remove etc. then it was shade dried till a constant weight was obtained. Then plant material was reduced in size to a coarse powder by an electrically driven grinder. Then it was soaked in 70% aqueous-ethanolic solution in glass beaker for three days. Occasional stirring of plant material was carried out during this time period and then it was filtered, filtrate was preserved in refrigerator in air tight container while marc was again soaked for next three days and same process was performed to obtain filtrate. Both filtrates were combined and dried by using rotary evaporator under reduced pressure and temperature. At end a dark brownish semisolid paste of stem and light brown paste of fruits was obtained. Percentage yield was calculated stem: 10% and fruits 22% [22-23].

Preliminary phytochemical evaluation: Primary phytochemical evaluation was performed for various secondary plant metabolites which show that they contain saponins, alkaloids, tannins, pholobatannins, flavanoids, steroids and glycosides [14,24-25].

Micro-organisms and media: Three gram positive bacteria *Staphylococcus aureus, Bacillus subtilis* and *Salmonella typhi* and three gram negative bacteria *Eschericha coli, Klebsilla pneumonia* and *Pseudomonas auregenosa* were selected for study. All bacterial cultures were clinical isolates which were identified by typical standard procedures. Two fungal strains *Cunninghamella echinulata and Aspergillus niger* were also chosen for evaluation of anti-fungal activity of plant extracts. All bacterial strains were grown in Nutrient broth (Merck, Germany) and fungal strains were grown in Sabourad's dextrose agar (Lab M, England).

Determination of antimicrobial activity by agar disc diffusion method: Antimicrobial activity of crude plant extracts was checked by using paper disc diffusion method [26-27]. All bacterial strains were adjusted to 0.5 McFarland turbidity standards before evaluating antimicrobial activity. Similarly before assessing antifungal activity both fungal isolates were adjusted to 10^6 cfu/ml. Bacterial strains ware inoculated on 15 cm sterilized nutrient agar Petri plates, while fungal cultures were seeded on sterile Sabourad's dextrose agar on 15cm diameter Petri plates. Plant extracts were dissolved in solvent at concentration of 10 mg, 20 mg, 40 mg and 80 mg per milliliter, then sterile filter paper discs whose diameters were 6 mm ware prepared and impregnated with 20 µl of plant extracts so that each disc contains 200 µg, 400 µg, 800 µg and 1600 µg of reconstituted plant extract respectively. These discs were placed on Petri plates with flamed forceps and pressed smoothly so that it has proper contact with agar, these Petri plates were formerly inoculated with bacterial and fungal isolates. Ciprofloxacin (Liofilchem, Italy) 5 µg

and Gentamicin (Liofilchem, Italy) 10 µg discs were placed which serves as positive antibacterial control and discs contains only solvent serves as negative control. In same way Fluconazole discs 400 µg serve as positive antifungal and only solvent disc serves as negative antifungal control. Bacterial cultures were incubated for 24 hours at 37°C while fungal cultures were grown at 36°C for 48 hours. After incubation period zone of inhibition diameter were measured in mm around discs. All these bioassays were performed thrice.

Determination of Minimum Inhibitory Concentration (MIC) by using 96-wells plates: 96-well plates under sterile conditions were used to determine antibacterial activity. The principle behind the method is that as number of bacterial cells increase during the lag phase of their life cycle the turbidity of the nutrient broth increases in which the bacteria are grown and therefore absorbance of the nutrient broth also increases [28-30]. For study three gram positive (*Staphylococcus aureus, Bacillus subtilis, Salmonella typhi)* and three gram negative bacteria *(Escherichia coli, Klebsilla pneumonia, Pseudomonas auregenosa*) were utilized. All the bacterial cultures were maintained on the nutrient broth solution and they were adjusted to 0.5 McFarland turbidity standards [31]. All test samples were dissolved in appropriate solvents. 20 µl of samples were pipetted into the wells at a concentration of 25-200 µg/ml. Then 180 µl of 12 hours old bacterial culture was added after suitable dilutions with nutrient broth into each well. Before incubation of the 96-wells micro plates the initial absorbance was adjusted among 0.12-0.19 at 540 nm. The final volume in each of the well was maintained to 200 µl. All bacterial cultures were incubated in incubator at 37°C with lids on micro titter plates for a period of 24 hours. Synergy HT Bio Tek® USA, micro plate reader was used to measure absorbance of the bacterial cultures before and after incubation period at 540 nm. The difference was taken as index of bacterial growth. Using the following formula percentage inhibition was calculated:

Percentage inhibition = (X – Y) 100 / X

Whereas: X = Absorbance of control with bacterial culture

Y = Absorbance of bacterial culture and test sample(s)

Results were calculated as mean of triplicate (n = 3 ± SEM). Ciprofloxacin and Gentamicin were taken as standard drugs for comparison, EZ – Fit5 Perrella Scientific Inc. Amherst USA software was used for calculation of the results, results were represented as MIC_{50}. Minimum inhibitory concentration (MIC) is that concentration of drug that inhibits any visible microbial growth. MIC_{50} – MIC_{90} are the concentrations which inhibit of 50% and 90% of bacterial growth.

RESULTS AND DISCUSSION

Prosopis cinerarium is extensively used for treatment of skin diseases, boils and as blood purifier [16]. Antimicrobial agents can be obtained from the plants as more than 1000 plants with

antimicrobial activity have been reported [32]. Therefore, antimicrobial activity of *Prosopis cineraria* (L) Druce leaves stems and fruits extract was performed individually which were prepared in 70% ethanol. Antimicrobial activity was assessed at concentration of 200, 400, 800 and 1600 µg/disc, by using disc diffusion method. Both stem and fruit extracts of *Prosopis cineraria* shows strong antimicrobial activity against all bacterial and fungal strains used during study.

Stem extracts of *Prosopis cineraria* inhibited the growth of all bacterial strains which were used during the study. The maximum zone of inhibition against *E. coli* was 23.3 mm, *P. auregenosa* 25.7 mm *S. aureus* 24.0 mm, *K. pneumonia* 25.3 mm, *B. subtilis* 30.0 mm and against *S. typhi* was 30.3 mm in diameter as shown in table 1, when these zones of inhibitions were determined at a dose of 1600 µg/disc. And when zone of inhibitions were measured at lowest concentration used during the study i.e. at 200 µg/disc, the zone of inhibition was against *E. coli* is 9.67 mm, *P. auregenosa* 9.33 mm *S. aureus* 10.0 mm, *K. pneumonia* 9.67 mm, *B. subtilis* 10.0 mm and against *S. typhi* was 10.3 mm. Saponins among the constituents may be one of the factors responsible for difference in antimicrobial activity against different strains i.e. gram positive and gram negative bacteria. Saponins may not be able to penetrate the cell membranes of gram negative bacteria. The effectiveness of saponins from *Prosopis cineraria* on gram-positive bacteria such as *B. subtilis* and *S. typhi*, may be as a result of difference in microbial cell wall. Modes of action of antibacterial activity of saponins against both gram-negative and gram-positive bacteria are not yet clear [33].

Table 1: Zone of inhibitions of *Prosopis Cineraria* stem extract and standard against various strains of bacteria

No	Strains of Bacteria	Diameter of zone of inhibition (mm) (Mean ± SEM)					
		Negative Control	Ciprofloxacin 5 µg	*Prosopis cineraria* 200 µg	*Prosopis cineraria* 400 µg	*Prosopis cineraria* 800 µg	*Prosopis cineraria* 1600 µg
1	*E. coli*	6.33 ± 0.333	31.7 ± 1.45***	9.67 ± 1.20***	18.7 ± 1.20**	20.3 ± 2.03*	23.3 ± 2.03*
2	*P. auregenosa*	6.33 ± 0.333	33.7 ± 2.91***	9.333 ± 0.88**	19.7 ± 1.45*	20.7 ± 1.67*	25.7 ± 1.76ns
3	*S. aureus*	6.33 ± 0.333	31.0 ± 1.53***	10.0 ± 1.53***	18.0 ± 1.73**	22.3 ± 2.03*	24.0 ± 1.73*
4	*K. pneumonia*	6.33 ± 0.333	34.3 ± 1.86***	9.67 ± 1.45***	20.0 ± 1.73**	22.0 ± 1.73**	25.3 ± 1.33*
5	*B. subtilis*	6.33 ± 0.333	34.3 ± 1.86***	10.0 ± 1.73***	18.3 ± 1.45**	22.0 ± 1.73**	30.0 ± 1.73ns
6	*S. typhi*	6.33 ±	36.7 ± 1.76***	10.3 ±	21.3 ±	23.3 ±	30.3 ±

	0.333	1.45***	2.60**	1.86**	2.03ns

n = 3. P < 0.05 significant (*), P < 0.01 significant (**), and P < 0.0001 highly significant (***), ns = non significant

Table 2: Zone of inhibitions of *Prosopis Cineraria* fruits extract and standard against various strains of bacteria

No	Strains of Bacteria	Diameter of zone of inhibition (mm) (Mean ± SEM)					
		Negative Control	Ciprofloxacin 5 µg	*Prosopis cineraria* 200 µg	*Prosopis cineraria* 400 µg	*Prosopis cineraria* 800 µg	*Prosopis cineraria* 1600 µg
1	*E. coli*	6.33 ± 0.333	31.7 ± 1.45***	8.00 ± 1.15***	19.0 ± 1.53**	22.3 ± 1.45*	23.3 ± 1.86*
2	*P. auregenosa*	6.33 ± 0.333	33.7 ± 2.91***	14.0 ± 1.15**	18.7 ± 1.20**	20.7 ± 2.60*	25.7 ± 1.45ns
3	*S. aureus*	6.33 ± 0.333	31.0 ± 1.53***	14.3 ± 0.882**	19.3 ± 1.86*	22.3 ± 2.03*	25.0 ± 2.31ns
4	*K. pneumonia*	6.33 ± 0.333	34.3 ± 1.86***	7.33 ± 0.88***	17.0 ± 2.08**	23.0 ± 2.31*	24.3 ± 2.40*
5	*B. subtilis*	6.33 ± 0.333	34.3 ± 1.86***	9.00 ± 0.57***	19.0 ± 1.15**	21.0 ± 2.65*	25.7 ± 1.76*
6	*S. typhi*	6.33 ± 0.333	36.7 ± 1.76***	6.33 ± 0.33***	18.0 ± 1.53**	21.7 ± 1.45**	25.0 ± 2.08*

n = 3. P < 0.05 significant (*), P < 0.01 significant (**), and P < 0.0001 highly significant (***), ns = non significant

At maximum *Prsopis cineraria* fruits extract concentration (1600 µg/disc), zone of inhibition against *E. coli, P. auregenosa*, *S. aureus*, *K. pneumoniae, B. subtilis, S. typhi* were 23.3 mm, 25.7 mm, 25.0 mm , 24.3 mm, 25.7 mm and 25.0 mm and zone of inhibition of extract at concentration of 200 µg/disc against *P. auregenosa* was 14.0 mm, against *S. aureus* was 14.3 mm and against *B. subtilis* was 9.0 mm while against *S. typhi, E. coli* and *K. pneumonia* fruit extract were found to be inactive at this concentration a shown in table 2. Antimicrobial activity of fruit extract might be related to their phenolic compounds, because plants synthesize phenolic compounds in response to microbial infection. It is therefore, possible that they can act as effective antimicrobial substances against a wide array of microorganisms. However, the antimicrobial activity of plant fruit extracts depends not only on phenolic compounds but also by the presence of different secondary metabolite. This might have synergistic effect, because of the ability of these substances to bind to bacterial adhesion [8].

At 1600 µg/disc concentration of *Prosopis cineraria* stem and fruit extracts against *Cunninghamella echinulata zones* of inhibition were 15.7 mm for each extract and against *Aspergillus niger* 17.00 mm and 18.7 mm, respectively as shown in table 3. While there is no growth inhibition in 200µg/disc used during study against each strain. Hypothetical increase in the antifungal activity of any extract of *Prosopis cineraria* supposed to be found by increase concentration (µg/ml). Nayan and Shukla in 2011 reported antifungal activity of leaves extract containing saponins, tannins, alkaloids, flavonoids, steroids and glycosides against *A.niger* with different concentrations showing increased antifungal activity with increase in concentration [34].

Table 3: Zone of inhibitions of *Prosopis Cineraria* stem extract and control against various strains of fungi

No	Strains of fungus	Diameter of zone of inhibition (mm) (Mean ± SEM)					
		Negative Control	Fluconazole 400 µg	*Prosopis cineraria* 200 µg	*Prosopis cineraria* 400 µg	*Prosopis cineraria* 800 µg	*Prosopis cineraria* 1600 µg
1	*Aspergillus niger*	6.33 ± 0.333	25.0 ± 1.15***	6.33 ± 0.333***	14.0 ± 1.15**	16.3 ± 1.20**	17.0 ± 1.15**
2	*Cunninghamella echinulata*	6.67 ± 0.333	16.7 ± 0.882***	7.00 ± 0.577***	12.0 ± 1.15*	14.7 ± 0.667ns	15.7 ± 1.45ns

n = 3. P < 0.05 significant (*), P < 0.01 significant (**), and P < 0.0001 highly significant (***), ns = non significant

Table 4: Zone of inhibitions of *Prosopis Cineraria* fruit extract and control against various strains of fungi

No	Strains of fungus	Diameter of zone of inhibition (mm) (Mean ± SEM)					
		Negative Control	Fluconazole 400 µg	*Prosopis cineraria* 200 µg	*Prosopis cineraria* 400 µg	*Prosopis cineraria* 800 µg	*Prosopis cineraria* 1600 µg
1	*Aspergillus niger*	6.33 ± 0.333	25.0 ± 1.15***	6.67 ± 0.333***	13.7 ± 0.882**	17.3 ± 0.882**	18.7 ± 1.20*
2	*Cunninghamella echinulata*	6.67 ± 0.333	16.7 ± 0.882***	6.33 ± 0.333***	13.3 ± 0.882ns	14.7 ± 0.882ns	15.7 ± 0.882ns

n = 3. P < 0.05 significant (*), P < 0.01 significant (**), and P < 0.0001 highly significant (***), ns = non significant

Table 5 and 6 indicated that all the extracts exerted inhibitory effect on the test organisms to different extent. Minimum inhibitory concentration which inhibits growth of 50% and 90% bacteria i.e. IC_{50} and IC_{90} values for leaves extract were 11.27 µg/ml & 14.17 µg/ml for *K. pneumonia,* 12.14 µg/ml & 16.0 µg/ml against *E. coli,* 11.16 µg/ml & 18.11 µg/ml for *P. auregenosa,* 12.0 µg/ml & 14.27 µg/ml for *S. aureus,* and 17.12 µg/ml & 17.14 µg/ml for *B. subtilis* and 13.11 µg/ml & 18.22 µg/ml for *S. typhi*. IC_{50} and IC_{90} values for stem was found to be 13.29 µg/ml & 19.41 µg/ml against *E. coli, 19.22* µg/ml & 21.51 µg/ml for *P. auregenosa,* 11.06 µg/ml & 21.16 µg/ml for *K. pneumonia,* 14.41 µg/ml & 23.12 µg/ml for *S. aureus,* 16.16 µg/ml & 15.29 µg/ml for *B. subtilis* was 16.51 µg/ml & 19.26 µg/ml for *S. typhi*. MIC_{50} and MIC_{90} values for fruit extract was 13.01 µg/ml & 18.62 µg/ml against *E. coli,* 17.06 µg/ml & 19.02 µg/ml for *P. auregenosa,* 19.13 µg/ml & 19.23 µg/ml for *K. pneumonia,* 15.19 µg/ml & 18.06 µg/ml for *K. pneumonia,* 13.71 µg/ml & 21.22 µg/ml for *B. subtilis* and 17.12 µg/ml & 19.11 µg/ml for *S. typhi.* The results obtained in the agar diffusion plates followed the same trend with what was obtained in the minimum inhibitory tests. Gram-negative bacteria were more sensitive than Gram-positive bacteria due to the presence of secondary metabolites such as alkaloids, flavonoids and steroids [35]. This is in agreement with previous reports that plant extracts are more active against Gram positive bacteria than Gram negative bacteria. These differences may be attributed to the fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall is multilayered structure.

Table 5: Minimum inhibitory concentration in µg / ml (MIC_{50})

No	Extract and Standard drugs	*E. coli*	*P. auregenosa*	*K. pneumonia*	*S. aureus*	*B. subtilis*	*S. typhi*
1	*Prosopis cineraria* leaves extract	12.14± 0.16	11.16 ±0.10	11.27±0.32	12.01± 0.70	17.12± 0.31	13.11± 0.38
2	*Prosopis cineraria stem extract*	13.29± 0.25	19.22±0.14	11.06±0.70	14.41± 0.42	16.16± 0.31	16.51± 0.22
3	*Prosopis cineraria* fruits extract	18.62± 0.22	17.06 ±0.24	19.13±0.04	15.19± 0.41	13.71± 0.18	17.12± 0.31
4	Ciprofloxacin	10.36± 0.3	8.42± 0.123	9.31 ±0.18	8.21± 0.02	11.21± 0.1	10.89± 0.11
5	Gentamicin	8.36 ± 0.12	9.42 ± 0.11	15.34±0.22	8.21 ± 0.02	7.59 ± 0.11	11.03± 0.10

Table 6: Minimum inhibitory concentration in µg / ml (MIC_{90})

No	Extract and Standard drugs	*E. coli*	*P. auregenosa*	*K. pneumonia*	*S. aureus*	*B. subtilis*	*S. typhi*
1	*Prosopis cineraria* leaves extract	16.01± 0.20	18.11±0.18	14.17±0.12	14.27± 0.70	17.14± 0.21	18.22± 0.11
2	*Prosopis cineraria stem extract*	19.41± 0.42	21.51±0.12	21.16±0.70	23.12± 0.04	15.29± 0.15	19.26± 0.11
3	*Prosopis cineraria* fruits extract	13.01± 0.41	19.02±0.11	19.23±0.14	18.06± 0.14	21.22± 0.02	19.11± 0.12
4	Ciprofloxacin	8.21 ± 0.02	11.03 ± 0.10	12.34±0.22	10.12 ± 0.11	12.16± 0.22	7.59 ± 0.11
5	Gentamicin	11.21± 0.12	12.19±0.21	11.11 ±0.08	13.42 ± 0.13	11.36± 0.3	14.21± 0.60

CONCLUSION:

Results of bioassay clearly indicate that aqueous ethanolic extracts of leaves and fruits of *Prosopis cineraria* possess antimicrobial properties which are possibly due to its secondary metabolites e.g. flavanoids, tannins, alkaloids and saponins etc. these results confirm its traditional uses for skin diseases, boils and as blood purifier etc.

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REFERENCES

1. Parekh J, Jadeja D and Chanda S. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. Turkish J of Biology, 2005; 29(4): 203-210.

2. Khan R, Islam B, Akram M, Shakil S, Ahmad A, Ali SM, Siddiqui M and Khan AU. Antimicrobial activity of five herbal extracts against multidrug resistant (MDR) strains of bacteria and fungus of clinical origin. Molecules, 2009; 14(2): 586-597.

3. Napar AZ, Bux H, Zia MA, Ahmad MZ, Iqbal A, Roomi S, Muhammad I and Shah SH. Antimicrobial and antioxidant activities of *Mimosaceae* plants; *Acacia modesta* wall (Phulai),

Prosopis cineraria (Linn.) and *Prosopis juliflora* (Swartz). J of Medicinal Plants Res, 2012; 6(15): 2962-2970.

4. Baris O, Gulluce M, Sahin F, Ozer H, Kilic H, Ozkan H, Sokmen M and Ozbek T. Biological activities of the essential oil and methanol extract of *Achillea Biebersteinii* Afan. (Asteraceae). Turkish J of bio, 2006; 30(4): 65-73.

5. Nostro A, Germano MP, Angelo VD, Marino A and Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Letters in Applied Microbio, 2000; 30(5): 379-384.

6. Velmurugan V, Arunachalam G and Ravichandran V. Anticonvulsant activity of methanolic extract of *Prosopis Cineraria* (Linn) Druce stems barks. Int J of Pharmaceutical Tech Res, 2012; 4(1): 89-92.

7. Dharani B, Sumathi S, Sivaprabha J and Padama PR. Invitro antoxidant potential of *Prosopis cineraria* leaves. J of Natural Products and Plant Resources, 2011; 1(3): 26-32.

8. Vijayakumar A, Duraipandiyan V, Jeyaraj B, Agastian P, Raj MK and Ignacimuthu S. Phytochemical analysis and in vitro antimicrobial activity of Illicium griffithii Hook. f. & Thoms extracts. Asian Pacific J of Tropical Dis, 2012; 2(3): 190-199.

9. Alanis AJ. Resistance to antibiotics: Are we in the Post-Antibiotic Era. Archives of Med Res, 2005; 36(6): 697-705.

10. Basri DF and Fan SH. The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agents. Indian J of pharmacol, 2005; 37(1): 26-29.

11. Narayan GR, Kartik V, Manoj P, Singh PS and Alka G. Antibacterial activities of ethanolic extracts of plants used in folk medicine. Int J of Research in Ayurveda & Pharmacy, 2010; 1(2): 529-535.

12. Fabry W, Okemo PO and Ansorg R. Antbacterial effects of East African medicinal plants. J of Ethanopharmacol, 1998; 60(1): 79-84.

13. Baquar SR. Medicinal and Poisonous plants of Pakistan. 1st ed. Printas Karachi, Pakistan, 1989: p. 21-45.

14. Evans WC. Trease and Evans Pharmacognosy. 15th ed. Saunders Elsevier Philadelphia, USA, 2004; p. 25-26, 336.

15. Velmurugan V, Arunachalam G and Ravichandran V. Anthelmintic potential of *Prosopis cineraria* (Linn.) Druce stem barks. Asian J of Plant Sci and Res, 2011; 1(2): 88-91.

16. Singh V and Pandey RP. Ethnobotany of Rajasthan. 1st Ed. Scientific Publishers. Jodhpur, India, 1998; p. 368.

17. Kirtikar KR and Basu BD. Indian medicinal plants. 2nd ed.vol III. Periodical expert book agency. Dehradun, India, 1994; p. 1655-1956.

18. Trivedi PC. Medicinal plants utilization and conservation. 1st ed. Aavishkar publishers and distributors, Jaipur 302003 (Raj), India, 2004; p. 262.

19. Singh V and Pandey RP. Economic and medicinal plants of Indian desert, In: Desert resources and technology. (Ed), Singh, Alam. Vol, I, Scientific publishers. Jodhpur, India, 1983; p. 307-368.

20. Khatri A, Rathore A and Patil UK. *Prosopis cineraria* (L.) Druce : A bone plant of desert an overview. Int J of Biomed and Advance Res, 2010; 1(5): 141-149.

21. Verma N, Sohal RK, Gupta R and Saraf SA. Formulation and evaluation of herbal depilatory cream. Pharmacologyonline, 2011; 3(1): 674-683.

22. Jabeen Q, Bashir S, Lyoussi B and Gillani AH. Coriander fruit exhibits gut modulatory, blood pressure lowering and diuretic activities. J of Ethanopharmacol, 2009; 122(1): 660-667.

23. Gilani AH, Jabeen Q, Ghayur MN, Janbaz KH and Akthar MS. Studies on the antihypertensive, antispasmodic, bronchodilator and hepatoprotective activities of the *Carum copticum* seed extract. J of Ethanopharmacol, 2005; 98(1): 127-135.

24. Abhishek M, Kumar DV, Kumar VS and Kumar SS. Phytochemical evaluation and in-vitro antioxidant activity of some medicinaly important plants of uttarakhand. Int Research J of Pharmacy, 2011; 2(6): 116-122.

25. Edeoga HO, Okwu DE and Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Africian J of Biotech, 2005; 4(7): 685-688.

26. Salama HMH and Marraiki N, Antimicrobial activity and phytochemical analysis of *Polygonum aviculare* L. (Polygonaceae), naturally grown in Egypt. Australian J of Basic and Applied Sci, 2009; 3(3): 2008-2015.

27. Ali MS, Al-nuri MA, Yaghmour RMR and Faidi YR. Antimicrobial activity of *Micromeria nervosa* from Palestinian area. J of Ethanopharmacol, 1997; 58(3): 143-147.

28. Cos P, Vlietinc AJ, Berghe DV and Maes L. Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'. J of Ethanopharmacol, 2006; 106(3): 290-302.

29. Kaspady M, Narayanaswamy VK, Raju M and Rao GK. Synthesis, Antibaterial activity of 2,4-Disubstituted Oxazoles and Thiazoles as Bioisosters. Letters in Drug Design and Discovery, 2009; 6(1): 21-28.

30. Patel AK, Pate RJ, Patel KH and Patel RM. Synthesis, characterization, thermal properties and antimicrobial activity of acrylic copolymers derived from 2, 4-dichlorophenyl acrylate. J of Chilean Chemical Society, 2009; 54(3): 228-233.

31. Rani P and Khullar N. Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant *Salmonella typhi.* Phytotherapy Res, 2004; 18(8): 670-673.

32. Nychas GJE. Natural antimicrobials from plants. In: Gould GW (Editor). New methods of food preservation. 1st ed. Blackie Acedemic and professional, London, 1995; p. 58-89.

33. Soetan k, Oyekunle M, Aiyelaagbe O and Fafunso M. Evaluation of the antimicrobial activity of saponins extract of *Sorghum Bicolor L. Moench*. African J of Biotech, 2006; 5(23): 2405-2407.

34. Nayan R, Bhalodia and Shukla VJ. Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* l.: An ethnomedicinal plant. J of Advanced Pharmaceutical Tech, 2011; 2: 104-109.

35. Varahalarao and Vadlapudi. In vitro antimicrobial activity of plant extracts of Avicennia alba against some important pathogens. Asian Pacific J of Tropical Disease, 2012; 2(5): 408-411.